



A chlorambucil-containing bioconjugate can be synthesized by the following methods. Chlorambucil is a relatively stable nitrogen mustard with attenuated alkylating ability, presumably as a consequence of the less-basic aniline nitrogen.

5 Method One: In this procedure, chlorambucil is converted to the acid chloride followed by reaction with cob(I)alamin or Co(I)[SALEN] according to reaction sequence I. In situations where the acyl linkage to the organocobalt complex is too labile towards serum nucleophiles, two alternate bioconjugation procedures can be utilized.

10 Method Two: The procedure involves bromination of a carbon atom adjacent to the carboxyl group under standard Hell-Vollhardt-Zelinski conditions to permit attachment of the Co complex in the  $\alpha$ -position according to reaction sequence II. In scheme II, replacement of the C-Co with C-H provides chlorambucil. The reactant stoichiometry, temperature, and dilutions conditions can be manipulated to avoid competing displacement of one of the chloroethyl groups, or of the C1 by  $S_N2$  attack.

15 Method Three: The BOC-protected p-aminophenylacetaldehyde can be conjugated to the Co moiety, followed by formation of the active nitrogen mustard product according to the following reaction sequence III.

